

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Rice et al.	Group No.:	1648
Serial No.:	09/576,989	Atty. Docket No.:	56029-4356
Filed:	05/23/2000		
For:	HCV Variants	Examiner:	Wortman, Donna C.

**DECLARATION OF DR. KERIL J. BLIGHT UNDER 37 C.F.R. §1.131**

I, Dr. Keril J. Blight, declare and state as follows:

1. All of the statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true.

2. I am a co-inventor of U.S. Patent Application No. 09/576,989 for HCV Variants, filed May 23, 2000 (Patent Application).

3. I, along with co-inventor Dr. Charles M. Rice (hereinafter, "we"), conceived of, and reduced to practice the inventions claimed in the Patent Application before March 31, 2000.

4. Specifically, before March 31, 2000 we identified the adaptive mutations that are described in the Patent Application. Those adaptive mutations are referenced in the attached laboratory notebook pages and computer printouts attached as Exhibit A. The terminology used to describe the cell colonies harboring HCV comprising those mutations in Exhibit A (see A25) corresponds to the terminology used in the Patent Application (see Figure 7) as follows:

BBI	HCVrep1b/Ava.1
BBII	HCVrep1b/Ava.5
BBIII	HCVrep1b/Huh.2
BBIV	HCVrep1b/Ava.7
BBV	HCVrep1b/Ava.2
BBVI	HCVrep1b/Clone A
BBVII	HCVrep1b/Clone B

5. Because the cell colonies were G418 resistant, we expected that the resistance was conferred by HCV replicons comprising adaptive mutations, harbored by those colonies. We tested this theory by sequencing the replicons, which were amplified from cDNA reverse transcribed from RNA isolated from each of the independent G418 resistant cell clones, before March 31, 2000. That data is presented at A3-A19. We then engineered each mutation back into the HCVrep1bBartMan/Avall backbone, as described in the Example in the Patent Application. We then transcribed RNA from each reconstructed replicon and electroporated it into naïve Huh7 cells, and compared the number of G418 resistant colonies compared to that obtained for the HCVrep1bBartMan/Avall replicon containing wild type NS5A (see A1, for example, where it was determined that the mutation identified in clone BBI was capable of increasing the frequency of G418 resistant colonies). Based on that result, we reasonably expected that the other mutations identified would similarly confer increased frequency of G418 resistant colonies, due to increased transfection efficiency of the mutant HCV.

7. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

\_\_\_\_\_  
Dr. Keril J. Blight

August \_\_, 2004

Electroporation Huh7B p60

1µg vs 10µg — WT/Ava.I  
 + 9µg — pol<sup>+</sup>/Ava.I  
 Huh7B — Original (HCVrepBarMan/Ava.I)  
 cellular RNA(4-1-00) — 10µg cellular RNA only

- \* 10 T175's split 1:2 ~ 24hr prior to electroporation
- \* Procedure as always (Plated 1/3 & 2/3 on p150's. Also removed 0.5ml of G18 for p60)
- \*  $4.2 \times 10^7$  cells total — Resuspend 3ml D-PBS  $\Rightarrow 5.5 \times 10^6$  cells/eq
- \* 24hr post-electroporation add G418 at 1mg/ml
- \* Trypsinize p60 dishes & seed on 8 well chamber slides for IF (no G418)
- \* Acetone fix ~ 8hr post-seeding
  - \* Plated remainder of cells (mg/ml) on a p120. Added G418 ~ 16hr after seeding
- $\Rightarrow$  IF for NS3 (H7)  $\Rightarrow$  NEGATIVE!

$\Rightarrow$  The deletion is adaptive

Electroporation Huh7 (CMR) p49

1µg vs 10µg — WT/Ava.I  
 + 9µg — pol<sup>+</sup>/Ava.I  
 Huh7B — Original (HCVrepBarMan/Ava.I)  
 cellular RNA(4-1-00) — 10µg cellular RNA only

- \* 8 T175's split 1:2 25hr prior to electroporation
- \* Procedure as previously (Plated 1/3 & 2/3 on p150's. Also removed 0.5ml from 1ml for p60)
- \*  $9.2 \times 10^7$  cells total  $\Rightarrow$  Resuspend 6ml PBS  $\Rightarrow 6 \times 10^6$  cells/EP
- \* 24hr post-electroporation add G418 at 0.8mg/ml

$\Rightarrow$  Deletion is adaptive in CMR Huh7's. In fact, more colonies are consistently observed for HCVrepBarMan & HCVrep/Ava.I in CMR Huh7 cells vs Bortnischlager's.

Big Dye SeqClone A  
~40ng PCR product

KB	Label	CMR #	Volume
486	A.1	#829	2 $\mu$ l
487	A.1	#862	
488	A.1	#869	
489	A.2	#884	
490		#885	
491		#1038	
492		#1039	
493	A.15	#1039	1 $\mu$ l
494		#1038	
495		#949	
496		#950	
497		#970	
498		#982	
499		#971	
500		#1030	
501	A.12	#1042	1 $\mu$ l
502		#931	
503		#1047	
504		#936	
505		#923	
506		#8990	
507	A.10	#1040	1.2 $\mu$ l
508		#1047	
509		#936	
510		#931	
511		#923	
512	A.13	#1029	4 $\mu$ l

KB	CMR #	PCR #
574	#983	#15
575	#1019	A.15
576	#1022	A.15
577	#1023	A.15
578	#819	A.20
579	#1030	A.15

Reamplified the gel  
purified product  
14-2-00  
except KB578-A.2

Tuesday,

Untitled

Construction parameters:

Match Size	12
Maximum Added Gap Length in Contig	70
Maximum Added Gap Length in Sequence	70
Minimum Match Percentage	65
Maximum Register Shift Difference	70
Last group Considered	2
Gap Penalty	0.00
Gap Length Penalty	0.70
Consensus Threshold	75

Clone A

CREATING NEW contig 1: from Hartman WT(1>8012)

ENTERING 01-KB486(12>691) in contig 1: percent match 99

ENTERING 02-KB487(33>650) in contig 1: percent match 94

ENTERING 03-KB488(36>444) in contig 1: percent match 99

ENTERING 04-KB489(18>657) in contig 1: percent match 98

ENTERING 05-KB490(26>629) in contig 1: percent match 95

ENTERING 06-KB491(1>516) in contig 1: percent match 98

ENTERING 07-KB492(8>848) in contig 1: percent match 95

ENTERING 08-KB493(1>520) in contig 1: percent match 97

ENTERING 09-KB494(2>740) in contig 1: percent match 97

ENTERING 10-KB495(1>742) in contig 1: percent match 96

ENTERING 11-KB496(21>786) in contig 1: percent match 98

ENTERING 12-KB497(20>737) in contig 1: percent match 96

ENTERING 13-KB498(4>843) in contig 1: percent match 96

ENTERING 14-KB499(1>661) in contig 1: percent match 93

ENTERING 15-KB500(11>779) in contig 1: percent match 97

ENTERING 16-KB501(1>112) in contig 1: percent match 92

ENTERING 17-KB502(17>791) in contig 1: percent match 98

ENTERING 18-KB503(1>752) in contig 1: percent match 99

ENTERING 19-KB504(2>737) in contig 1: percent match 97

ENTERING 20-KB505(21>720) in contig 1: percent match 98

ENTERING 21-KB506(8>757) in contig 1: percent match 98

ENTERING 22-KB507(1>633) in contig 1: percent match 98

ENTERING 23-KB508(1>728) in contig 1: percent match 98

ENTERING 24-KB509(7>745) in contig 1: percent match 98

ENTERING 25-KB510(18>703) in contig 1: percent match 99

ENTERING 26-KB511(1>711) in contig 1: percent match 94

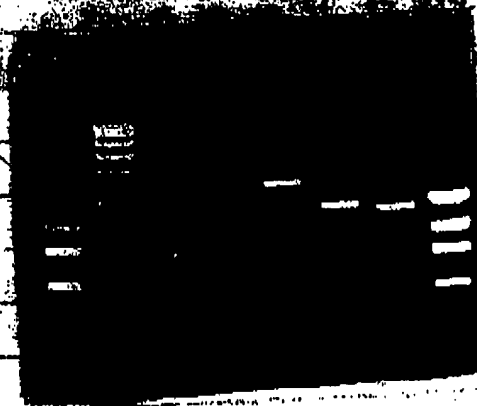
Sequence 27-KB512 was not added, it is all poor data

Elapsed Time 0:0:16



- \* Separate PCR products on 0.8% TBE
- \* Isolate DNA from gel slices (as on 5-2-00)

- \* Pellet purified DNA & wash 2x 80% EtOH
- \* Resuspend 10µl TE
- \* Quantitate 0.5µl



Big Dye Seq ~ 100ng PCR product / 0.8µl oligo @ 4pmol/µl

KB513	CMR #862	PCR #1	KB528	CMR #1029	PCR #3	KB571	CMR
514	#867		529	#1022	PCR #3	572	CMR
515	#829		530	#1030	PCR #3	573	CMR
516	#884	PCR #2	531	#1040	PCR #4		
517	#885		532	#1047			
518	#1038		533	#936			
519	#1039		534	#931			
520	#1038	PCR #3	535	#923			
521	#1039		536	#1047	PCR #5		
522	#949		537	#936			
523	#950		538	#931			
524	#910		539	#923			
525	#971		540	#1042			
526	#482		541	#899c			



Wednesday,

Untitled

Construction parameters:

Match Size	12
Minimum Added Gap Length in Contig	70
Minimum Added Gap Length in Sequence	70
Minimum Match Percentage	65
Maximum Register Shift Difference	70
Lastgroup Considered	2
Gap Penalty	0.00
Gap Length Penalty	0.70
Consensus Threshold	75

Avo.1

CREATING NEW contig 1: from BartMan WT copy(1>8012)

ENTERING 01.KB513(16>572) in contig 1: percent match 96

ENTERING 02.KB514(33>443) in contig 1: percent match 99

ENTERING 03.KB515(1>600) in contig 1: percent match 99

ENTERING 04.KB516(1>806) in contig 1: percent match 95

ENTERING 05.KB517(5>698) in contig 1: percent match 94

ENTERING 06.KB518(1>518) in contig 1: percent match 98

ENTERING 07.KB519(14>821) in contig 1: percent match 94

ENTERING 08.KB520(6>711) in contig 1: percent match 96

ENTERING 09.KB521(11>514) in contig 1: percent match 98

ENTERING 10.KB522(14>666) in contig 1: percent match 97

ENTERING 11.KB523(11>756) in contig 1: percent match 97

ENTERING 12.KB524(22>729) in contig 1: percent match 95

ENTERING 13.KB525(24>734) in contig 1: percent match 92

ENTERING 14.KB526(1>707) in contig 1: percent match 97

ENTERING 15.KB527(19>853) in contig 1: percent match 91

ENTERING 16.KB528(23>535) in contig 1: percent match 85

ENTERING 17.KB529(43>658) in contig 1: percent match 97

ENTERING 18.KB530(42>775) in contig 1: percent match 89

ENTERING 19.KB531(7>679) in contig 1: percent match 98

ENTERING 20.KB532(19>723) in contig 1: percent match 98

ENTERING 21.KB533(1>859) in contig 1: percent match 96

ENTERING 22.KB534(1>750) in contig 1: percent match 97

ENTERING 23.KB535(17>755) in contig 1: percent match 98

ENTERING 24.KB536(8>829) in contig 1: percent match 97

ENTERING 25.KB537(1>763) in contig 1: percent match 96

ENTERING 26.KB538(12>697) in contig 1: percent match 99

ENTERING 27.KB539(18>739) in contig 1: percent match 99

ENTERING 28.KB540(22>114) in contig 1: percent match 89

ENTERING 29.KB541(25>692) in contig 1: percent match 98

Elapsed Time 0:0:18

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PAGE 18/37 \* RCVD AT 8/30/2004 6:12:40 PM [Eastern Daylight Time] \* SVR:USPTO-EFXRF-1/5 \* DNIS:8729306 \* CSID: \* DURATION (mm-ss):11-12

8.2 8.3 A.15 100ng DNA

B.2 80ng/μl

B.3 80ng/μl

A.15 120ng/μl

Big Dye Seq ~ 40ng PCR product

KB542	CMR #862	B.1	KB560	CMR #1040	B.4
543	#867	B.1	561	#1047	
544	#879	B.1	562	#936	
545	#884	B.2	563	#931	
546	#885		564	#923	
547	#1038		565	#1047	B.5
548	#1039		566	#936	
549	#1038	B.3	567	#931	
550	#1039		568	#923	
551	#949		569	#1042	
552	#950		570	#899c	
553	#970				
554	#971				
555	#982				
556	#983				
557	#1029				
558	#1022				
559	#1030				

Thursday,  
Untitled

Construction parameters:  
Match Size 12  
Maximum Added Gap Length in Contig 70  
Maximum Added Gap Length in Sequence 70  
Minimum Match Percentage 65  
Maximum Register Shift Difference 70  
Lastgroup Considered 2  
Gap Penalty 0.00  
Gap Length Penalty 0.70  
Consensus Threshold 75

Clone B

CREATING NEW contig 1: from BartMan WT copy(1>8012)  
ENTERING 31-KB542(41>556) in contig 1: percent match 99  
ENTERING 32-KB543(34>444) in contig 1: percent match 99  
ENTERING 33-KB544(3>597) in contig 1: percent match 99  
ENTERING 34-KB545(1>690) in contig 1: percent match 95  
ENTERING 35-KB546(1>598) in contig 1: percent match 96  
ENTERING 36-KB547(1>515) in contig 1: percent match 97  
ENTERING 37-KB548(1>574) in contig 1: percent match 95  
ENTERING 38-KB549(12>678) in contig 1: percent match 98  
ENTERING 39-KB550(29>512) in contig 1: percent match 98  
ENTERING 40-KB551(15>620) in contig 1: percent match 97  
ENTERING 41-KB552(7>676) in contig 1: percent match 98  
ENTERING 42-KB553(12>594) in contig 1: percent match 98  
ENTERING 43-KB554(2>597) in contig 1: percent match 93  
ENTERING 44-KB555(1>696) in contig 1: percent match 98  
ENTERING 45-KB556(5>707) in contig 1: percent match 96  
ENTERING 46-KB557(6>567) in contig 1: percent match 95  
ENTERING 47-KB558(37>700) in contig 1: percent match 96  
ENTERING 48-KB559(18>596) in contig 1: percent match 96  
ENTERING 49-KB560(2>646) in contig 1: percent match 97  
ENTERING 50-KB561(7>662) in contig 1: percent match 97  
ENTERING 51-KB562(1>677) in contig 1: percent match 98  
ENTERING 52-KB563(1>693) in contig 1: percent match 97  
ENTERING 53-KB564(16>725) in contig 1: percent match 97  
ENTERING 54-KB565(1>695) in contig 1: percent match 98  
ENTERING 55-KB566(8>687) in contig 1: percent match 98  
ENTERING 56-KB567(1>600) in contig 1: percent match 99  
ENTERING 57-KB568(15>732) in contig 1: percent match 97  
ENTERING 58-KB569(14>127) in contig 1: percent match 85  
ENTERING 59-KB570(7>686) in contig 1: percent match 98  
Elapsed Time 0:0:17

Thursday,  
Project: Untitled Contig 1

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46-KB557(5>567)	5270	5280	5290	5300	5310	5320	5330	5340	5350
48-KB559(18>536)	5360	5370	5380	5390	5400	5410	5420	5430	5440
BartMan WT copy(1>8012)	5450	5460	5470	5480	5490	5500	5510	5520	5530
46-KB557(5>567)	5540	5550	5560	5570	5580	5590	5600	5610	5620
48-KB559(18>596)	5630	5640	5650	5660	5670	5680	5690	5700	5710
BartMan WT copy(1>8012)	5720	5730	5740	5750	5760	5770	5780	5790	5800
46-KB557(5>567)	5810	5820	5830	5840	5850	5860	5870	5880	5890
48-KB559(18>596)	5900	5910	5920	5930	5940	5950	5960	5970	5980
BartMan WT copy(1>8012)	5990	6000	6010	6020	6030	6040	6050	6060	6070
46-KB557(5>567)	6080	6090	6100	6110	6120	6130	6140	6150	6160
48-KB559(18>596)	6170	6180	6190	6200	6210	6220	6230	6240	6250
BartMan WT copy(1>8012)	6260	6270	6280	6290	6300	6310	6320	6330	6340
46-KB557(5>567)	6350	6360	6370	6380	6390	6400	6410	6420	6430
48-KB559(18>596)	6440	6450	6460	6470	6480	6490	6500	6510	6520
BartMan WT copy(1>8012)	6530	6540	6550	6560	6570	6580	6590	6600	6610
46-KB557(5>567)	6620	6630	6640	6650	6660	6670	6680	6690	6700
48-KB559(18>596)	6710	6720	6730	6740	6750	6760	6770	6780	6790
BartMan WT copy(1>8012)	6800	6810	6820	6830	6840	6850	6860	6870	6880
46-KB557(5>567)	6890	6900	6910	6920	6930	6940	6950	6960	6970
48-KB559(18>596)	6980	6990	7000	7010	7020	7030	7040	7050	7060
BartMan WT copy(1>8012)	7070	7080	7090	7100	7110	7120	7130	7140	7150
46-KB557(5>567)	7160	7170	7180	7190	7200	7210	7220	7230	7240
48-KB559(18>596)	7250	7260	7270	7280	7290	7300	7310	7320	7330
BartMan WT copy(1>8012)	7340	7350	7360	7370	7380	7390	7400	7410	7420
46-KB557(5>567)	7430	7440	7450	7460	7470	7480	7490	7500	7510
48-KB559(18>596)	7520	7530	7540	7550	7560	7570	7580	7590	7600
BartMan WT copy(1>8012)	7610	7620	7630	7640	7650	7660	7670	7680	7690
46-KB557(5>567)	7700	7710	7720	7730	7740	7750	7760	7770	7780
48-KB559(18>596)	7790	7800	7810	7820	7830	7840	7850	7860	7870
BartMan WT copy(1>8012)	7880	7890	7900	7910	7920	7930	7940	7950	7960
46-KB557(5>567)	7970	7980	7990	8000	8010	8020	8030	8040	8050
48-KB559(18>596)	8060	8070	8080	8090	8100	8110	8120	8130	8140
BartMan WT copy(1>8012)	8150	8160	8170	8180	8190	8200	8210	8220	8230
46-KB557(5>567)	8240	8250	8260	8270	8280	8290	8300	8310	8320
48-KB559(18>596)	8330	8340	8350	8360	8370	8380	8390	8400	8410
BartMan WT copy(1>8012)	8420	8430	8440	8450	8460	8470	8480	8490	8500
46-KB557(5>567)	8510	8520	8530	8540	8550	8560	8570	8580	8590
48-KB559(18>596)	8600	8610	8620	8630	8640	8650	8660	8670	8680
BartMan WT copy(1>8012)	8690	8700	8710	8720	8730	8740	8750	8760	8770
46-KB557(5>567)	8780	8790	8800	8810	8820	8830	8840	8850	8860
48-KB559(18>596)	8870	8880	8890	8900	8910	8920	8930	8940	8950
BartMan WT copy(1>8012)	8960	8970	8980	8990	9000	9010	9020	9030	9040
46-KB557(5>567)	9050	9060	9070	9080	9090	9100	9110	9120	9130
48-KB559(18>596)	9140	9150	9160	9170	9180	9190	9200	9210	9220
BartMan WT copy(1>8012)	9230	9240	9250	9260	9270	9280	9290	9300	9310
46-KB557(5>567)	9320	9330	9340	9350	9360	9370	9380	9390	9400
48-KB559(18>596)	9410	9420	9430	9440	9450	9460	9470	9480	9490
BartMan WT copy(1>8012)	9500	9510	9520	9530	9540	9550	9560	9570	9580
46-KB557(5>567)	9590	9600	9610	9620	9630	9640	9650	9660	9670
48-KB559(18>596)	9680	9690	9700	9710	9720	9730	9740	9750	9760
BartMan WT copy(1>8012)	9770	9780	9790	9800	9810	9820	9830	9840	9850
46-KB557(5>567)	9860	9870	9880	9890	9900	9910	9920	9930	9940
48-KB559(18>596)	9950	9960	9970	9980	9990	10000	10010	10020	10030
BartMan WT copy(1>8012)	10040	10050	10060	10070	10080	10090	10100	10110	10120
46-KB557(5>567)	10130	10140	10150	10160	10170	10180	10190	10200	10210
48-KB559(18>596)	10220	10230	10240	10250	10260	10270	10280	10290	10300
BartMan WT copy(1>8012)	10310	10320	10330	10340	10350	10360	10370	10380	10390
46-KB557(5>567)	10400	10410	10420	10430	10440	10450	10460	10470	10480
48-KB559(18>596)	10490	10500	10510	10520	10530	10540	10550	10560	10570
BartMan WT copy(1>8012)	10580	10590	10600	10610	10620	10630	10640	10650	10660
46-KB557(5>567)	10670	10680	10690	10700	10710	10720	10730	10740	10750
48-KB559(18>596)	10760	10770	10780	10790	10800	10810	10820	10830	10840
BartMan WT copy(1>8012)	10850	10860	10870	10880	10890	10900	10910	10920	10930
46-KB557(5>567)	10940	10950	10960	10970	10980	10990	11000	11010	11020
48-KB559(18>596)	11030	11040	11050	11060	11070	11080	11090	11100	11110
BartMan WT copy(1>8012)	11120	11130	11140	11150	11160	11170	11180	11190	11200
46-KB557(5>567)	11210	11220	11230	11240	11250	11260	11270	11280	11290
48-KB559(18>596)	11300	11310	11320	11330	11340	11350	11360	11370	11380
BartMan WT copy(1>8012)	11390	11400	11410	11420	11430	11440	11450	11460	11470
46-KB557(5>567)	11480	11490	11500	11510	11520	11530	11540	11550	11560
48-KB559(18>596)	11570	11580	11590	11600	11610	11620	11630	11640	11650
BartMan WT copy(1>8012)	11660	11670	11680	11690	11700	11710	11720	11730	11740
46-KB557(5>567)	11750	11760	11770	11780	11790	11800	11810	11820	11830
48-KB559(18>596)	11840	11850	11860	11870	11880	11890	11900	11910	11920
BartMan WT copy(1>8012)	11930	11940	11950	11960	11970	11980	11990	12000	12010
46-KB557(5>567)	12020	12030	12040	12050	12060	12070	12080	12090	12100
48-KB559(18>596)	12110	12120	12130	12140	12150	12160	12170	12180	12190
BartMan WT copy(1>8012)	12200	12210	12220	12230	12240	12250	12260	12270	12280
46-KB557(5>567)	12290	12300	12310	12320	12330	12340	12350	12360	12370
48-KB559(18>596)	12380	12390	12400	12410	12420	12430	12440	12450	12460
BartMan WT copy(1>8012)	12470	12480	12490	12500	12510	12520	12530	12540	12550
46-KB557(5>567)	12560	12570	12580	12590	12600	12610	12620	12630	12640
48-KB559(18>596)	12650	12660	12670	12680	12690	12700	12710	12720	12730
BartMan WT copy(1>8012)	12740	12750	12760	12770	12780	12790	12800	12810	12820
46-KB557(5>567)	12830	12840	12850	12860	12870	12880	12890	12900	12910
48-KB559(18>596)	12920	12930	12940	12950	12960	12970	12980	12990	13000
BartMan WT copy(1>8012)	13010	13020	13030	13040	13050	13060	13070	13080	13090
46-KB557(5>567)	13100	13110	13120	13130	13140	13150	13160	13170	13180
48-KB559(18>596)	13190	13200	13210	13220	13230	13240	13250	13260	13270
BartMan WT copy(1>8012)	13280	13290	13300	13310	13320	13330	13340	13350	13360
46-KB557(5>567)	13370	13380	13390	13400	13410	13420	13430	13440	13450
48-KB559(18>596)	13460	13470	13480	13490	13500	13510	13520	13530	13540
BartMan WT copy(1>8012)	13550	13560	13570	13580	13590	13600	13610	13620	13630
46-KB557(5>567)	13640	13650	13660	13670	13680	13690	13700	13710	13720
48-KB559(18>596)	13730	13740	13750	13760	13770	13780	13790	13800	13810
BartMan WT copy(1>8012)	13820	13830	13840	13850	13860	13870	13880	13890	13900
46-KB557(5>567)	13910	13920	13930	13940	13950	13960	13970	13980	13990
48-KB559(18>596)	14000	14010	14020	14030	14040	14050	14060	14070	14080
BartMan WT copy(1>8012)	14090	14100	14110	14120	14130	14140	14150	14160	14170
46-KB557(5>567)	14180	14190	14200	14210	14220	14230	14240	14250	14260
48-KB559(18>596)	14270	14280	14290	14300	14310	14320	14330	14340	14350
BartMan WT copy(1>8012)	14360	14370	14380	14390	14400	14410	14420	14430	14440
46-KB557(5>567)	14450	14460	14470	14480	14490	14500	14510	14520	14530
48-KB559(18>596)	14540	14550	14560	14570	14580	14590	14600	14610	14620
BartMan WT copy(1>8012)	14630	14640	14650	14660	14670	14680	14690	14700	14710
46-KB557(5>567)	14720	14730	14740	14750	14760	14770	14780	14790	14800
48-KB559(18>596)	14810	14820	14830	14840	14850	14860	14870	14880	14890
BartMan WT copy(1>8012)	14900	14910	14920	14930	14940	14950	14960	14970	14980
46-KB557(5>567)	14990	15000	15010	15020	15030	15040	15050	15060	15070
48-KB559(18>596)	15080	15090	15100	15110	15120	15130	15140	15150	15160
BartMan WT copy(1>8012)	15170	15180	15190	15200	15210	15220	15230	15240	15250
46-KB557(5>567)	15260	15270	15280	15290	15300	15310	15320	15330	15340
48-KB559(18>596)	15350								

Big Dye Seq ~ 40ng PCR product Ava. 5

Ava. 5 PCR #1 30ng/μl  
 PCR #2 170ng/μl  
 PCR #3 150ng/μl  
 PCR #4 60ng/μl  
 PCR #5 30ng/μl

iscalculated  
 Added  
 5ng PCR  
 robot, instead  
 of 40ng

KB580	CMR #862	PCR #1
581	#867	↓
582	#829	↓
583	CMR #884	PCR #2
584	#885	↓
585	#1038	↓
586	#1039	↓
587	#1014	↓
588	CMR #1038	PCR #3
589	#1039	↓
590	#949	↓
591	#950	↓
592	#970	↓
593	#971	↓
594	#982	↓
595	#983	↓
596	#1029	↓
597	#1022	↓
598	#1030	↓
599	#1023	↓

KB600	CMR #1040	PCR #4
601	#1047	↓
602	#936	↓
603	#931	↓
604	#923	↓
605	#1047	PCR #5
606	#936	↓
607	#931	↓
608	#913	↓
609	#1042	↓
610	#849C	↓



Tuesday,

Untitled

Construction parameters:	12
Match Size	70
Minimum Added Gap Length in Contig	70
Minimum Added Gap Length in Sequence	65
Minimum Match Percentage	70
Maximum Register Shift Difference	2
Lastgroup Considered	0.00
Gap Penalty	0.70
Gap Length Penalty	75
Consensus Threshold	

Ava.5

CREATING NEW contig 1: from PartMan WF copy 1(1>8012)

ENTERING 01.KB580(33>533) in contig 1: percent match 97

ENTERING 02.KB581(1>439) in contig 1: percent match 94

ENTERING 03.KB582(16>598) in contig 1: percent match 90

Sequence 04.KB583 was not added, it is all poor data

Sequence 05.KB584 was not added, it is all poor data

Sequence 06.KB585 was not added, it is all poor data

NOT ENTERING in contig 1: 07.KB586(11>408) due to percent match (43) below threshold 65

NOT ENTERING in contig 1: 07.KB586(11>488) due to percent match (47) below threshold 65

CREATING NEW contig 2: from 07.KB586(11>488)

Sequence 08.KB587 was not added, it is all poor data

ENTERING 09.KB588(1>665) in contig 1: percent match 97

ENTERING 10.KB589(8>515) in contig 1: percent match 98

ENTERING 11.KB590(1>739) in contig 1: percent match 96

ENTERING 12.KB591(1>734) in contig 1: percent match 96

ENTERING 13.KB592(8>738) in contig 1: percent match 95

ENTERING 14.KB593(1>576) in contig 1: percent match 94

ENTERING 15.KB594(1>708) in contig 1: percent match 95

ENTERING 16.KB595(9>698) in contig 1: percent match 94

ENTERING 17.KB596(3>558) in contig 1: percent match 94

ENTERING 18.KB597(34>531) in contig 1: percent match 98

ENTERING 19.KB598(20>545) in contig 1: percent match 98

ENTERING 20.KB599(5>325) in contig 1: percent match 94

ENTERING 21.KB600(1>596) in contig 1: percent match 97

NOT ENTERING in contig 2: 22.KB601(1>701) due to percent match (51) below threshold 65

ENTERING 22.KB601(1>701) in contig 1: percent match 96

NOT ENTERING in contig 2: 23.KB602(1>540) due to percent match (50) below threshold 65

ENTERING 23.KB602(1>540) in contig 1: percent match 98

NOT ENTERING in contig 2: 24.KB603(6>648) due to percent match (45) below threshold 65

ENTERING 24.KB603(6>648) in contig 1: percent match 98

ENTERING 25.KB604(13>733) in contig 1: percent match 95

NOT ENTERING in contig 2: 26.KB605(1>735) due to percent match (51) below threshold 65

ENTERING 26.KB605(1>735) in contig 1: percent match 98

NOT ENTERING in contig 2: 27.KB606(1>603) due to percent match (50) below threshold 65

ENTERING 27.KB606(1>603) in contig 1: percent match 99

NOT ENTERING in contig 2: 28.KB607(1>703) due to percent match (45) below threshold 65

ENTERING 28.KB607(1>703) in contig 1: percent match 95

ENTERING 29.KB608(17>727) in contig 1: percent match 96

ENTERING 30.KB609(14>117) in contig 1: percent match 90

ENTERING 31.KB610(43>698) in contig 1: percent match 97

Elapsed Time 0:0:17

Tuesday,  
Project: Aya.5 align Config 1

Page 9

	4970	4980	4990	5000	5010	5020	5030	5040	5050	5060
19-KB598(20>545)										
15-KB594(1>708)										
18-KB597(34>551)										
17-KB596(3>558)										
19-KB598(20>545)										
BarEman WT copy 1(1>8012)										
18-KB597(34>551)										
17-KB596(3>558)										
19-KB598(20>545)										
BarEman WT copy 1(1>8012)										
17-KB596(3>558)										
19-KB598(20>545)										
20-KB599(5>325)										
BarEman WT copy 1(1>8012)										
17-KB596(3>558)										
19-KB598(20>545)										
20-KB599(5>325)										
BarEman WT copy 1(1>8012)										
17-KB596(3>558)										
19-KB598(20>545)										
20-KB599(5>325)										
BarEman WT copy 1(1>8012)										
17-KB596(3>558)										
19-KB598(20>545)										
20-KB599(5>325)										

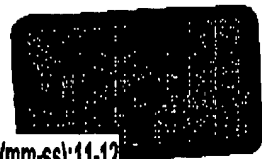


Big Dye Seq ~ 40ng PCR product Ava.2

Ava.2 PCR #1 20ng/μl  
 PCR #2 120ng/μl  
 PCR #3 80ng/μl  
 PCR #4 40ng/μl  
 PCR #5 70ng/μl

KB611	CMR#862	PCR #1	KB631	CMR#1040	PCR #4
612	#867	↓	KB632	#1041	↓
613	#829	↓	KB633	#936	↓
* 614	#884	PCR #2	KB634	#931	↓
* 615	#885	↓	KB635	#923	↓
* 616	#1038	↓	KB636	#1047	PCR #5
617	#1039	↓	637	#936	PCR #5
* 618	#1014	↓	638	#931	↓
619	#1038	PCR #3	639	#923	↓
620	#1039	↓	640	#1042	↓
621	#949	↓	641	#899c	↓
622	#950	↓			
623	#770	↓			
624	#971	↓			
625	#982	↓			
626	#983	↓			
627	#1029	↓			
628	#1022	↓			
629	#1030	↓			
630	#1023	↓			

\* Reactions 614-616 & 618 did not work → See Emily's comments





Wednesday,

Project: Unified Conting 1

Page 9

Barthman WT copy (1>8012)	5180	5190	5200	5210	5220	5230	5240	5250	5260
18-KB628(13>596)									
19-KB629(11>702)									
17-KB527(8>568)									
Barthman WT copy (1>8012)	5270	5280	5290	5300	5310	5320	5330	5340	5350
19-KB629(11>702)									
17-KB527(8>568)									
20-KB630(23>335)									
Barthman WT copy (1>8012)	5360	5370	5380	5390	5400	5410	5420	5430	5440
19-KB629(11>702)									
17-KB527(8>568)									
20-KB630(23>335)									
Barthman WT copy (1>8012)	5460	5470	5480	5490	5500	5510	5520	5530	5540
19-KB629(11>702)									
17-KB527(8>568)									
20-KB630(23>335)									
Barthman WT copy (1>8012)	5550	5560	5570	5580	5590	5600	5610	5620	5630
19-KB629(11>702)									
17-KB527(8>568)									
20-KB630(23>335)									
21-KB631(2>679)									
Barthman WT copy (1>8012)	5650	5660	5670	5680	5690	5700	5710	5720	5730
19-KB629(11>702)									
17-KB527(8>568)									
20-KB630(23>335)									
21-KB631(2>679)									

Wednesday.

Untitled

## Construction parameters:

Window Size	12
Minimum Added Gap Length in Contig	70
Minimum Added Gap Length in Sequence	70
Minimum Match Percentage	65
Maximum Register Shift Difference	70
Lastgroup Considered	2
Gap Penalty	0.00
Gap Length Penalty	0.70
Consensus Threshold	75

Ava. 2

CREATING NEW contig 1: from BartMan WT copy(1&gt;8012)

ENTERING 01-KB611(36&gt;680) in contig 1: percent match 95

ENTERING 02-KB612(35&gt;446) in contig 1: percent match 99

ENTERING 03-KB613(11&gt;666) in contig 1: percent match 99

Sequence 04-KB614 was not added, it is all poor data —

Sequence 05-KB615 was not added, it is all poor data —

Sequence 06-KB616 was not added, it is all poor data —

NOT ENTERING in contig 1: 07-KB617(1&gt;597) due to percent match (47) below threshold 65

NOT ENTERING in contig 1: 07-KB617(1&gt;597) due to percent match (51) below threshold 65

CREATING NEW contig 2: from 07-KB617(1&gt;597)

Sequence 08-KB618 was not added, it is all poor data

NOT ENTERING in contig 2: 09-KB619(16&gt;662) due to percent match (45) below threshold 65

ENTERING 09-KB619(16&gt;662) in contig 1: percent match 92

ENTERING 10-KB620(9&gt;517) in contig 1: percent match 96

ENTERING 11-KB621(65&gt;627) in contig 1: percent match 96

Sequence 12-KB622 was not added, it is all poor data —

ENTERING 13-KB623(38&gt;575) in contig 1: percent match 97

ENTERING 14-KB624(6&gt;414) in contig 1: percent match 92

ENTERING 15-KB625(1&gt;700) in contig 1: percent match 98

ENTERING 16-KB626(20&gt;542) in contig 1: percent match 95

ENTERING 17-KB627(8&gt;568) in contig 1: percent match 94

ENTERING 18-KB628(38&gt;696) in contig 1: percent match 96

ENTERING 19-KB629(11&gt;782) in contig 1: percent match 96

ENTERING 20-KB630(23&gt;335) in contig 1: percent match 95

ENTERING 21-KB631(2&gt;679) in contig 1: percent match 98

NOT ENTERING in contig 2: 22-KB632(1&gt;707) due to percent match (50) below threshold 65

ENTERING 22-KB632(1&gt;707) in contig 1: percent match 97

NOT ENTERING in contig 2: 23-KB633(1&gt;721) due to percent match (51) below threshold 65

ENTERING 23-KB633(1&gt;721) in contig 1: percent match 97

NOT ENTERING in contig 2: 24-KB634(7&gt;642) due to percent match (47) below threshold 65

ENTERING 24-KB634(7&gt;642) in contig 1: percent match 98

ENTERING 25-KB635(13&gt;726) in contig 1: percent match 97

NOT ENTERING in contig 2: 26-KB636(1&gt;680) due to percent match (50) below threshold 65

ENTERING 26-KB636(1&gt;680) in contig 1: percent match 98

NOT ENTERING in contig 2: 27-KB637(1&gt;688) due to percent match (50) below threshold 65

ENTERING 27-KB637(1&gt;688) in contig 1: percent match 97

NOT ENTERING in contig 2: 28-KB638(12&gt;658) due to percent match (45) below threshold 65

ENTERING 28-KB638(12&gt;658) in contig 1: percent match 98

ENTERING 29-KB639(15&gt;728) in contig 1: percent match 97

ENTERING 30-KB640(8&gt;135) in contig 1: percent match 85

ENTERING 31-KB641(1&gt;666) in contig 1: percent match 86

Elapsed Time 0:0:16

DNA Strider™ 1.317 ### Wednesday,

HCVrep1b BartMan/AvaII [1801 to 7758] -&gt; Translate - 1-frame

DNA sequence 11313 bp gccagaaaccca .7. cgaactcactata circular

artemischlager replicon I377/NS3-3'UTR (Genbank AJ242652).

Constructed in the pMT backbone.

Marked by AvaII in the variable region of the 3'UTR.

NS3

1801/1  
atg ggc cct att acg gcc tac tcc caa cag acg cga ggc cta ctt ggc tgc atc atc act  
M A V I T A Y S Q Q T R Q L L G Q I I T

1861/21  
agg ctc aca ggc cgg gac agg aac cag gtc gag ggg gag gtc caa gtc gtc tcc acc gca  
S L T Q R D R H Q V R G E V Q V V S T A

1921/41  
aca caa tct ttc ctg ggc acc tgc gtc aat ggc gtc tgt tgg act gtc tat cat ggt gcc  
T Q S F L A T Q V N G V C W T V Y H Q A

1981/61  
ggc tca aag acc ctt gcc ggc cca aag ggc cca atc acc caa atg tac acc aat gtc gac  
G S K T L A G E K G P I T Q M T T N V D

2041/81  
cag gac ctc gtc ggc tgc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc  
Q D L V G W Q A P P G A R S L R F C T C

2101/101  
ggc agc tgc gac ctt tac tgc ggc acc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc  
G S S D D T L V T N H A D V I P V R R R

2161/121  
ggc gac acc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc  
G D S R Q S L L S P R P V A Y L E Q S S

2221/141  
ggc ggc cca ctg ctc tgc ccc tgc ggc ccc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc  
G G D L L C P S Q G N A V G I F R A A V C

2281/161  
ggc  
R G V A K A V D F V P V H S M E T G M

2341/181  
ggc tcc cgc gtc ttc acc gac aac cgc tcc ccc ggc ggc gta cgc cgc acc ttc cag gtc  
K S F V P T D M B S P P A V P Q T F Q V

2401/201  
ggc cat cta caa gcc cct act ggc agc ggc aag agc act aag gtc cgc ggc tat gca  
A H L H A F P Q S G K S T K V P A A T A

2461/221  
ggc cca ggc tat aag gtc ctt gtc ctg aac cgc tcc gtc gcc gcc acc cta ggc ttc ggc  
A Q G Y K V L V L N P S V A A T L Q F G

2521/241  
ggc tat atg tct aag gca cat ggc acc gac cct aac atc aga ggc ggc gta agg acc acc  
A Y M S K A E Q I D P M I R T G V R T I

2581/261  
acc acc ggc gcc ccc atc acc tac tcc acc tat ggc aag ttt ctt gcc gac ggc ggc ggc  
G T Q A P I T T S T Y Q K P L A D G G C

2641/281  
tct ggc ggc gcc tat gac atc ata ata tgc gat gag tgc ccc tca acc gac tgc acc acc  
S G Q A T D I I I C D E C H S T D S T T

2701/301  
atc ctg ggc atc ggc aca gtc ctg gac caa ggc gag acc ggc ggc ggc ggc ggc ggc ggc ggc  
I L G I G T V L D Q A E T A G A R L V V

2761/321  
ctc gcc acc ggc acc ccc cgc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc ggc  
L A T A T P P Q S V T V P H P H I E E V

2821/341  
ggt ctg tcc agc act gga gaa atc ccc ttt tat ggc aag gcc atc ccc atc ggc acc acc  
A L S S T Q E I P P Y G K A I P I E T I

2881/361  
ggc  
G G G R H L I P C H S K K K C D E L A A

2941/381  
aag ctg tcc ggc ctc gga ctc aat ggc gta gca tat tgc ggc ggc ctt gat gta tcc gtc  
K L S G L G L H A V A Y Y R G L D V S V

3001/401  
atc cca act aac gga gac gcc att ttc gta gca aag gac ggc cta atg acc ggc ttt acc  
T T C A A C C G G A G C A T T T C G T A G A A G G C C T A T G A C C G C T T A C

Ava.11/Clone C/Clone D  
n.t. 5336Ava.13 n.t. 5320  
Ava.7 n.t. 5313  
Huh.2 n.t. 5314Clone A  
cga → ggc 87  
Gln ArgE → G 177  
GAA GGAT → I 255  
ACC ATC

CloneAva.5  
ag<sup>+</sup> → gg<sup>+</sup>  
Ser Gly

Huh. 5:

$D = 958 \text{ siles}$   
 $1172/1177/1179$

Clone Hh. 4  
Clone Ava. 11  
Clone C d D  
Clone A. # B  
agg → ctc  
Ser Ile

Clone Ava  
agg → gg  
Arg Gl

gcc → tcc  
Ala Ser  
clone Ava. 2  
Ava. 13

AA7 aa Clone Ava.I  
n.45345-5485

ISDR (40aa)

① Amino acid changes  
cf 90con

$100 \rightarrow 200$   
 Ger      Pro

1147.2

1142

19-3-00

G418-colonies picked for BartMan/AvaII in CMR Huh7 cells

Huh.4 10 $\mu$ g.  $\frac{1}{3}$  8-2-00  
 Huh.5 1 $\mu$ g  $\frac{2}{3}$  "  
 Huh.6 1 $\mu$ g  $\frac{1}{3}$  "  
 Huh.7 1 $\mu$ g  $\frac{1}{3}$  "  
 Huh.8 10 $\mu$ g  $\frac{1}{3}$  "  
 Huh.9 10 $\mu$ g  $\frac{1}{3}$  "

⇒ G418 added to 750 $\mu$ g/ml on 20-3-00Huh.4

- Transfer to 24 well plate 21-3-00 p1

- Transfer to 6 well plate 22-3-00 p2

- Transfer to T25 flask 25-3-00 p3

ALSO, Cell count #1  $3.2 \times 10^5$  cells/ml }  $\sim 3 \times 10^5$  cells/ml  
 Cell count #2  $2.95 \times 10^5$  "

⇒ Trizol extract RNA from 80 $\mu$ l cells ( $2.4 \times 10^4$  cells)

- Transfer to T75 flask 30-3-00 p4

10-4-00 - Froze 12 vials @  $4 \times 10^6$  cells/vial Huh.4 p7 (split 1:2 8-4-00)

Tank 2 Rack 5 Box 1 (1 vial); Box 6 (1 vial); Box 7 (1 vial);

Box 8 (6 vials); Box 9 (1 vial) &amp; Tank 2 Rack 7 Box 3 (2 vials)

Huh.5

- Transfer to 24 well plate 24-3-00 p1

- Transfer to 12 well plate 28-3-00 p2

- Transfer to 6 well plate 30-3-00 p3

- Transfer to T25 flask 2-4-00 p4

ALSO, Cell count #1  $4.4 \times 10^5$  cells/ml }  $4.3 \times 10^5$  ⇒ Trizol extract RNA from  
 Cell count #2  $4.15 \times 10^5$  " } 80 $\mu$ l ( $\sim 3.4 \times 10^4$  cells)

- Transfer to T75 flask 4-4-00 p5

20-4-00 - Froze 10 vials @  $4 \times 10^6$  cells/vial p8 (3 T75s split 1:1.5 on 11-4-00)

Huh. 7

- Transfer to 24 well plate 22-3-00 p1
- Transfer to 12 well plate 25-3-00 p2
- Transfer to 6 well plate 26-3-00 p3
- Transfer to T25 flask 29-3-00 p4

ALSO, Cell count #1  $2.75 \times 10^5$  cells/ml }  $3 \times 10^5$  cells/ml  
 Cell count #2  $3.5 \times 10^5$  }

⇒ Trizol extract RNA from 80µl ( $2.4 \times 10^4$  cells)

- Transfer to T75 flask 2-4-00 p5

12-4-00 - Froze 12 vials @  $4 \times 10^6$  cells/vial Huh. 7 p8 (split 1:2 on 10-4-00)

Tank 2 Rack 7 Box 3 (2 vials)

" " Box 4 (7 vials)

" " Box 5 (3 vials)

Huh. 8

- Transfer to 24 well plate 21-3-00 p1
- Transfer to 12 well plate 24-3-00 p2
- Transfer to 6 well plate 26-3-00 p3
- Transfer to T25 flask 29-3-00 p4

ALSO, Cell count #1  $1.4 \times 10^5$  cells/ml }  $1.75 \times 10^5$  cells/ml  
 Cell count #2  $2 \times 10^5$  }

⇒ Trizol extract RNA from 85µl ( $1.5 \times 10^4$  cells)

- Transfer to T75 flask 3-4-00 p5

14-4-00 Froze 10 vials @  $4 \times 10^6$  cells/vial Huh. 8 p8 (split 1:2 12-4-00)

Tank 2 Rack 3 Box 2 (1 vial)

Box 4 (1 vial)

Box 5 (1 vial)

Box 6 (1 vial)

Tank 1 Rack 3 Box 4 (1 vial)

Box 8 (1 vial)

Remainder stored in -80°C freezer box



Hub.9

- Transfer to 24 well plate 28-3-00 p1  
 - Transfer to 12 well plate 6-4-00 p2 (media change 12-4-00)

- Transfer to 6 well plate 14-1-00 p3

- Transfer to T25 flask 19-4-00 p4

ALSO, Cell count #1  $6.9 \times 10^5$  cells/ml }  $\sim 8.5 \times 10^5$  cells/ml  
 Cell count #2  $10^6$  cells/ml }

→ Trizol extract RNA from 70  $\mu$ l ( $\sim 6 \times 10^4$  cells)

- Transfer to T75 flask 23-4-00 p5

10-5-00 - Freeze 12 vials at  $4 \times 10^6$  cells/vial p8

Stored -80°C freezer box

Clone D

- Transfer to 24 well plate p1

- Transfer to 12 well plate p2

- Transfer to 6 well plate p3

- Transfer to T25 flask 16-3-00 p4

ALSO, Cell count #1  $3.4 \times 10^5$  cells/ml }  $3 \times 10^5$  cells/ml  
 Cell count #2  $2.5 \times 10^5$  }

→ Trizol extract 80  $\mu$ l ( $2.7 \times 10^4$  cells)

- Transfer to T75 flask 20-3-00 p5

31-3-00 - Freeze 7 vials @  $3.5 \times 10^6$  cells/vial p8 (4 T75's split 1:2 on 29-3-00)

Tank 2 Rack 7 Box 7

20-4-00Electroporation Huh7(cmr) p69~  $6 \times 10^6$  cells/electroporation+ 1.5  $\mu$ g replicon RNA + 5  $\mu$ g Huh7B cellular RNA (4-1-00)

- (1) HCVrep/Ava.2 BB.V
- (2) HCVrep/Ava.5 BB.II
- (3) HCVrep/Clone B BB.VII
- (4) HCVrep/Ava.1 BB.I
- (5) HCVrep13/ $\Delta$ ISDR
- (6) HCVrep BartMao/Ava.II (original)
- (7) HCVrep(pol-)/Ava.1
- (8) Polio rep-GFP

Place electroporated cells into media. Total volume = 9.5ml

A. Plate 0.5ml / p100

B. Plate 3ml &amp; 6ml per p150

21-4-00

\* At G418 at 0.8mg/ml ~ 27hr post-electroporation

\* Stain p100's on 6-5-00

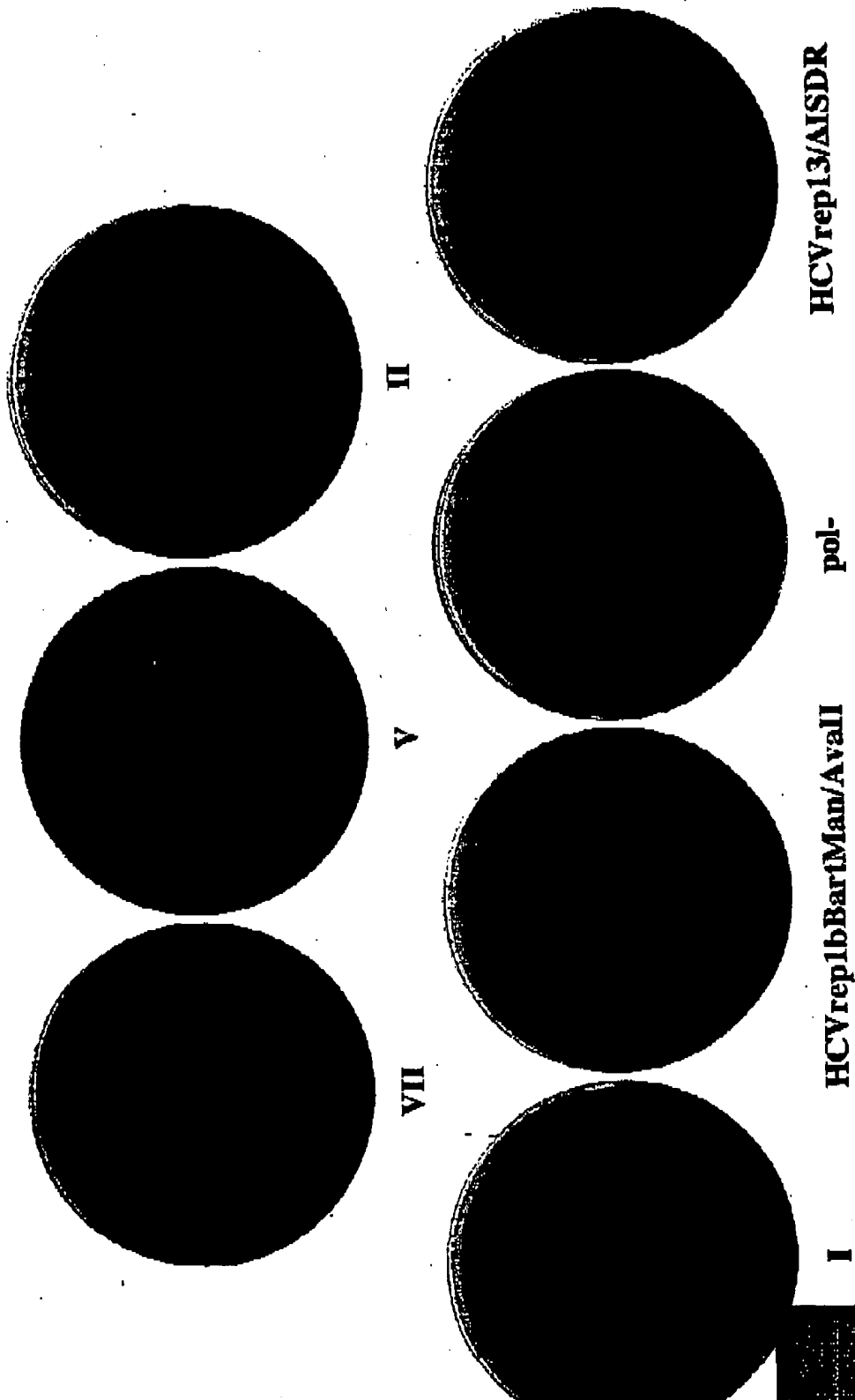
\* Stain p100 with 3ml plated on 14-5-00

N.B. When the cells are plated too dense they begin to detach due to overconfluence, particularly Clone B & Ava. 2 which appear to replicate in >90% of Huh7 cells

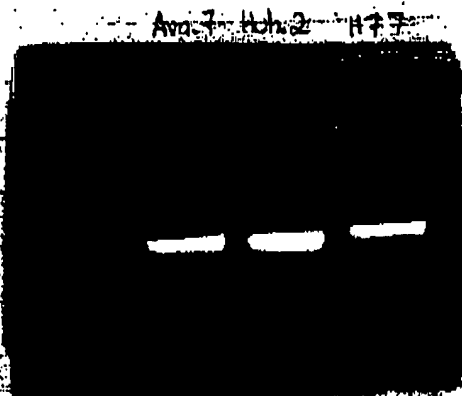
\* Clone B &gt; Ava. 2 &gt; Ava. 5 &amp; Ava. 1 # of G418-resistant colonies

\* No colonies observed for H77 HCVrep13/ $\Delta$ ISDR & HCVrep(pol-)/Ava. 1

**G418-resistant colonies (Experiment 20-4-00)**



Check  $1\mu\text{g}$  ( $\sim 2\mu\text{g}$ ) on non-denaturing gel



21-5-00

Electroporation Huh7 p69

5 T175b split 1:2 ~ 24hr prior

1.5  $\mu\text{g}$  HCVrep RNA + 4  $\mu\text{g}$  Huh7B cellular RNA (4-1-00)

$\sim 6 \times 10^6$  cells/electroporation

1. HCVrep1b/Ava.1 BB I
2. HCVrep1b/Ava.2 BB V
3. HCVrep1b/Ava.5 BB II
4. HCVrep1b/Ava.7 BB IV
5. HCVrep1b/Clone B BB VII
6. HCVrep1b/Huh.2 BB III
7. HCVrep1b/Ser  $\rightarrow$  Ile (1179)
8. HCVrep1b BartMan/Ava II (original)
9. HCVrep1b BartMan(pol<sup>-</sup>)/Ava II

Volume total  $\sim 9.5\text{ml}$   $\rightarrow$  Plate 0.4ml, 0.3ml, 0.2ml, 0.1ml per p100

Plate 1.5ml per p150

(N.B. Also plated 6ml/p150 for HCVrep1b/Ser  $\rightarrow$  Ile)

22-5-00Electroporation HeLa cells p15

- \* 5 T175's split 30hr prior 1:2
- \*  $1.3 \times 10^8$  cells total resuspended in 2.5ml ice-cold D-PBS
- \* 99  $\mu$ sec, 0.9 kV, 5 pulses, 0.4ml cells ( $\sim 6 \times 10^6$  cells)
- 2  $\mu$ g HCVrep RNA

- 1 HCVrep1b/Clone B BB VII
- 2 HCVrep1b/Ava.1 BB I
- 3 HCVrep1b/Ava.2 BB V
- 4 HCVrep1b/Ava.5 BB II
- 5 HCVrep1b/Ava.7 BB IV
- 6 HCVrep1b/Huh.2 BB III
- 7 HCVrep1b/BartMan/AvaII
- 8 HCVrep1b/BartMan(pol-)/AvaII
- 9 HCVrep13/S  $\rightarrow$  I
- 10 HCVrep13/ $\Delta$ ISDR
- 11 No RNA

\*  $V_T \sim 9.5$ ml media + EPed cells

\* Plate 3ml & 6ml per p150

" 0.5ml per p100 (For no RNA EP, plated total cells on p150)

24-5-00

At 48hr post-EP add 0.9 mg/ml G418

$\Rightarrow$  No colonies observed for any of the RNAs electroporated

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